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Spo13 Maintains Centromeric Cohesion and Kinetochore Coorientation during Meiosis I

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Summary

Background: The meiotic cell cycle, the cell division cycle that leads to the generation of gametes, is unique in that a single DNA replication phase is followed by two chromosome segregation phases. During meiosis I, homologous chromosomes are segregated, and during meiosis II, as in mitosis, sister chromatids are partitioned. For homolog segregation to occur during meiosis I, physical linkages called chiasmata need to form between homologs, sister chromatid cohesion has to be lost in a stepwise manner, and sister kinetochores must attach to microtubules emanating from the same spindle pole (coorientation).
Results: Here we show that the meiosis-specific factor Spo13 functions in two key aspects of meiotic chromosome segregation. In cells lacking *SPO13*, cohesin, which is the protein complex that holds sister chromatids together, is not protected from removal around kinetochores during meiosis I but is instead lost along the entire length of the chromosomes. We furthermore find that Spo13 promotes sister kinetochore coorientation by maintaining the monopolin complex at kinetochores. In the absence of *SPO13*, Mam1 and Lrs4 disassociate from kinetochores prematurely during pro-metaphase I and metaphase I, resulting in a partial defect in sister kinetochore coorientation in *spo13*Δ cells.
Conclusions: Our results indicate that Spo13 has the ability to regulate both the stepwise loss of sister chromatid cohesion and kinetochore coorientation, two essential features of meiotic chromosome segregation.

Introduction

Sexually reproducing organisms rely on a specialized cell cycle, the meiotic cell cycle, for the maintenance of ploidy in their progeny. The meiotic cell cycle consists of a single DNA replication phase followed by two chromosome segregation phases, thus producing haploid gametes. The diploid complement is then restored in the zygote, when the gametes from each parent are mated to each other. In the first meiotic division, the reductional division, homologs segregate away from each other, and in the second equational division, which resembles the mitotic division, sister chromatids separate (reviewed in [1–3]).

The reduction in chromosome number during meiosis I requires at least three processes that are unique to meiosis. First, homologous chromosomes from both parents must be physically linked to each other to allow for the proper attachment of the chromosomes to the meiosis I spindle. In most organisms these linkages are created by a process called reciprocal recombination, which leads not only to the joining of DNA molecules between the two homologs but also to the formation of a cytological structure called a chiasma (reviewed in [4]).

The second process necessary for the meiotic chromosome segregation program to occur accurately is the stepwise loss of cohesion between the sister chromatids. The cohesin complex that forms a ring around the sister chromatids mediates cohesion between sister chromatids during both mitosis and meiosis [5, 6]. In meiosis, one subunit of the cohesin complex, Scc1/Mcd1, is exchanged for a meiosis-specific homologous protein, Rec8, in most but not all areas of the genome [7–9]. Because the homologs are joined at the DNA level as a result of reciprocal recombination, cohesion not only links the sister chromatids but also binds all four homologous chromatids together. Thus, for homologs to segregate away from each other during meiosis I, cohesion distal to chiasmata must be removed. To accomplish this, cells remove cohesins along the entire arm of chromosomes at the onset of anaphase I. The removal of cohesins along chromosome arms at the onset of anaphase I is brought about by the proteolysis of Rec8, by a conserved protease called separase [10, 11]. However, unlike in mitosis, during which cohesins are removed along the entire chromosome at the metaphase-to-anaphase transition, in meiosis cohesins are retained around the centromere [8, 9]. This retention of centromeric cohesins is essential for accurate sister chromatid segregation during meiosis II. It ensures that sister chromatids stably attach to the meiosis II spindle and segregate accurately during anaphase II. Retention of centromeric cohesion requires a member of the MEI-S332 family of proteins. MEI-S332 was first identified in *Drosophila* as essential for preventing loss of centromeric cohesion during meiosis I and localizes to centromeric regions from prophase I until the onset of anaphase II [12–14]. Recently, proteins that are distantly related to MEI-S332 and which are termed shugoshins have been identified in both *S. pombe* and *S. cerevisiae* [15–18]. In both organisms, shugoshin, Sgo1, is essential for preventing the dissociation of cohesins from centromeric regions during meiosis I.

The third process necessary for bringing about homolog segregation during meiosis I is the attachment of sister chromatids to microtubules that emanate from the same spindle pole. Sister kinetochores are then said to be cooriented. This attachment is in contrast to mitosis and meiosis II, when sister chromatids bind to microtubules emanating from opposite poles and are bioriented (reviewed in [1–3]). Recently, a complex of proteins, the monopolin complex, was discovered in budding yeast, and this complex promotes sister kinetochore coorientation [19, 20]. This complex is composed

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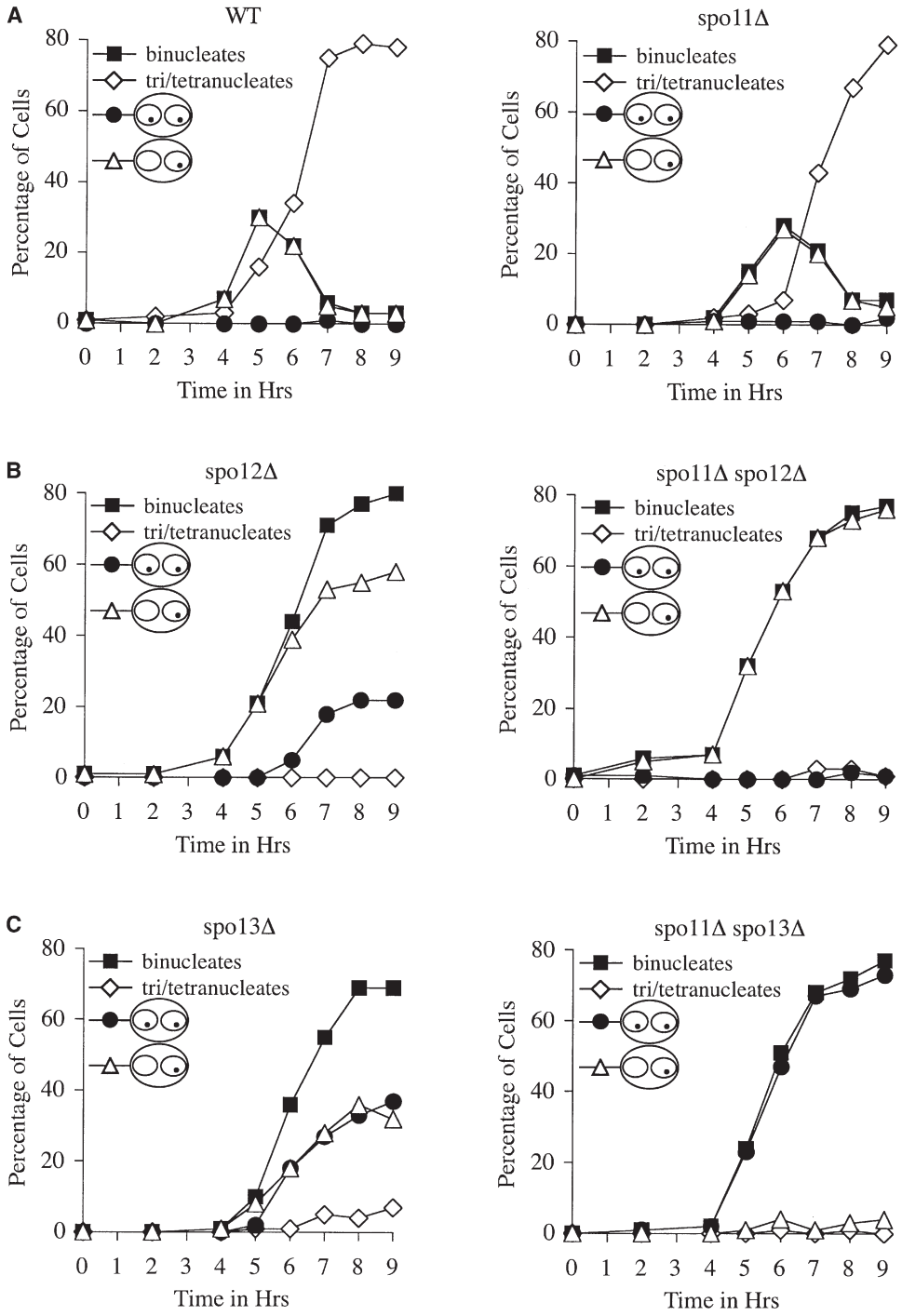
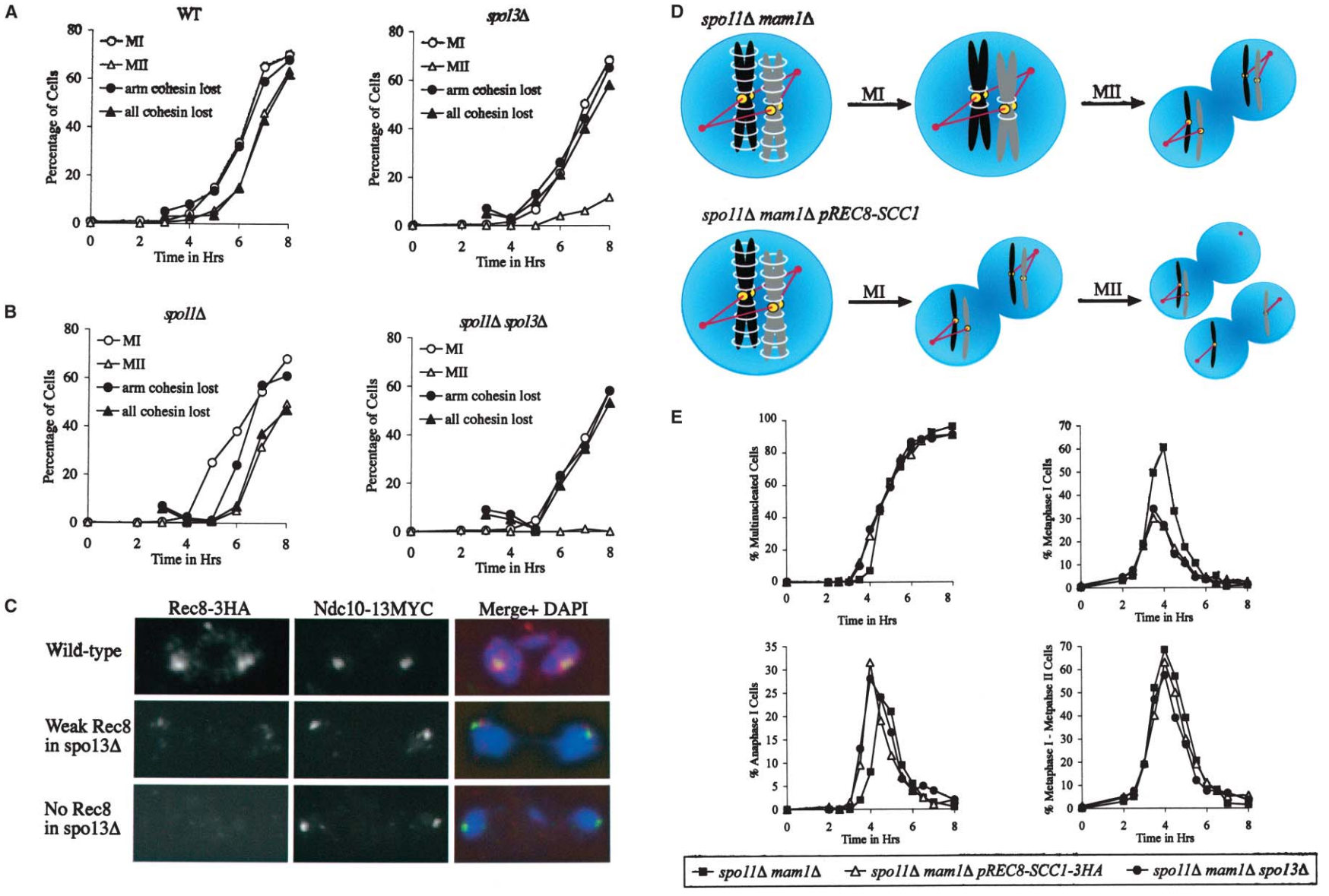


Figure 1. The *spo13*Δ Phenotype Is Distinct from that Exhibited by FEAR Network Mutants

Wild-type ([A] left, A5811), *spo11*Δ ([A] right, A7314), *spo12*Δ ([B] left, A5814), *spo11*Δ *spo12*Δ ([B] right, A9168), *spo13*Δ ([C] left, A5891), and *spo11*Δ *spo13*Δ ([C] right, A7170) cells carrying heterozygous CENV GFP dots were sporulated as described in the Experimental Procedures. The percentage of binucleates (closed squares), tri/tetranucleates (open diamond), binucleated cells with GFP label in only one of the two nuclei (open triangle; reductional), and binucleated cells with GFP label in both nuclei (closed circle; equational) was determined at the indicated time points.

of Mam1, Lrs4, and Csm1 and localizes to the kinetochores in late prophase and metaphase I. Cells lacking any of these proteins fail to coorient sister kinetochores during meiosis I and attempt to segregate sister chromatids instead of homologs during this division.

Meiosis I-specific events required for accurate meiotic chromosome segregation are regulated by meiosis-specific factors. One such meiosis-specific factor is *SPO13*. In 1980, Klapholz and Esposito identified *SPO13* as being required for the execution of two meiotic divisions


A
WT
spo13Δ
MI
MII
arm cohesin lost
all cohesin lost
Percentage of Cells
Time in Hrs
B
spo11Δ
spo11Δ spo13Δ
C
Rec8-3HA
Ndc10-13MYC
Merge+ DAPI
Wild-type
Weak Rec8 in spo13Δ
No Rec8 in spo13Δ
D
spo11Δ mam1Δ
MI
MII
spo11Δ mam1Δ pREC8-SCC1
E
% Multinucleated Cells
% Metaphase I Cells
% Anaphase I Cells
% Metaphase I - Metaphase II Cells
spo11Δ mam1Δ
spo11Δ mam1Δ pREC8-SCC1-3HA
spo11Δ mam1Δ spo13Δ

in budding yeast [21], and it is likely to be involved in regulating both the stepwise loss of cohesins from chromosomes and sister kinetochore coorientation. Cells lacking *SPO13* undergo a single meiotic division during which chromosomes exhibit a "mixed" segregation pattern; that is, some chromosomes exhibit a reductional meiosis I-like segregation pattern, whereas others segregate in a meiosis II-like, equational manner [22, 23]. Interestingly, in the absence of recombination, this mixed pattern is lost, and chromosomes segregate exclusively in an equational manner [24]. Furthermore, Rec8 is lost from centromeric regions in binucleated cells in *spo13*Δ mutants, and overexpression of *SPO13* during mitosis prevents cohesin cleavage and removal from chromosomes [8, 25, 26].

Here we investigate how Spo13 affects cohesion removal and whether the protein is involved in promoting sister kinetochore coorientation during meiosis I. Our analysis of the *spo13*Δ mutant indicates that *SPO13* regulates the retention of centromeric cohesins and suggests that retention of centromeric cohesin requires the cooperation of multiple pathways. Furthermore, we show that *SPO13* is required for maintaining monopolin complexes at kinetochores during metaphase I. Our results suggest that *SPO13* is a central regulator of meiosis I events. It controls retention of centromeric cohesion and kinetochore coorientation, both essential aspects of meiosis I chromosome segregation.

Results

The *spo13*Δ Phenotype Is Distinct from that of FEAR Network Mutants

Cells in which *SPO13* is deleted exhibit a terminal phenotype similar to that of cells defective for FEAR (Cdc fourteen early anaphase release) network function [22, 23, 27–30]. FEAR network mutants, like *spo13*Δ mutants, produce two-spored asci (dyads), have a delayed exit from anaphase I, and exhibit a mixed chromosome segregation pattern (Figure 1; [29, 30]). A detailed analysis of cells lacking FEAR network components revealed that these phenotypes are due to an uncoupling of meiotic events in these mutants [29, 30]. FEAR network mutants have a delayed exit from meiosis I. However, they nevertheless initiate meiosis II, resulting in both chromosome segregation phases occurring on the same meiotic spindle. This phenotype is most clearly observed when the occurrence of the reductional (meiosis I-like) and equational (meiosis II-like) chromosome segregation phase is analyzed in a synchronous meiotic cell cycle [29, 30]. One can determine chromosome segregation patterns by monitoring the segregation of green fluorescence protein (GFP)-labeled chromosome V (heterozygous CENV GFP dots; [31, 32]). In a reductional segregation, sister chromatids stay together so that a GFP dot is observed in just one nucleus in binucleated cells. However, if an equational segregation occurs, then sister chromatids separate and generate binucleated cells with GFP dots in both nuclei. When FEAR network mutants, such as a *spo12*Δ mutant, are examined by this assay, it is apparent that the equational segregation of chromosome V occurs after the reductional segregation during meiosis I, which is indicative of the uncoupling of meiotic events (Figure 1B; [29, 30]).

To determine whether the mixed chromosome segregation pattern exhibited by *spo13*Δ mutants is also due to an uncoupling of meiotic events, we determined the timing with which chromosome V segregated reductionally and equationally in a meiotic time course. In contrast to *spo12*Δ mutants, *spo13*Δ mutants produced binucleated cells with GFP dots in either one or both nuclei at the same time and with equal frequency, indicating that the reductional and equational segregation observed for chromosome V in *spo13*Δ mutants occur at the same time (Figures 1B and 1C). Furthermore, the cohesin subunit Rec8 is lost from centromeric regions at the same time as from chromosomal arms, consistent with *spo13*Δ mutants undergoing both reductional and

Figure 2. *SPO13* Is Required for Maintenance of Centromeric Cohesion

(A) Wild-type (A4758) and *spo13*Δ (A4837) cells carrying *REC8-3HA* and *NDC10-13MYC* fusions (*NDC10* encodes a kinetochore component) were sporulated as described. The percentage of cells with two or more nuclei (open circles) or three and four nuclei (open triangles) was determined by 4′,6-diamidino-2-phenylindole (DAPI) staining. The percentage of Ndc10-positive cells with Rec8 absent from chromosome arms (closed circles) or with no Rec8 staining (closed triangles) was determined by meiotic spreads.

(B) *spo11*Δ (A7240) and *spo11*Δ *spo13*Δ (A7810) cells carrying *REC8-3HA* and *NDC10-13MYC* fusions (*NDC10* encodes a kinetochore component) were sporulated and processed as described in Figure 2A.

Note: *spo13*Δ and *spo11*Δ *spo13*Δ cells are slightly delayed in entering meiosis I, as evidenced by the appearance of metaphase I spindles (Figure S1); thus, Rec8 is likely to be lost during meiosis I in *spo13*Δ cells rather than meiosis I despite their disappearance coinciding with meiosis II in wild-type cells.

(C) Examples of Rec8 localization in wild-type (top) cells and *spo13*Δ mutants (middle and bottom) during anaphase I; Rec8 is shown in red, Ndc10 in green, and DNA in blue. Note that the partial Rec8 staining observed in *spo13*Δ mutants was enhanced with Photoshop so that it would be visible in the photograph.

(D) Model of chromosome segregation in *spo11*Δ *mam1*Δ mutant. A pair of sister chromatids (black) and its homolog (gray) are held together by cohesin rings (white). The kinetochores (yellow) of the sister chromatids are attached to microtubules (red) in a bipolar manner. During meiosis I, arm cohesins are lost, but centromeric cohesins prevent chromosome segregation (top middle panel). The meiotic cell cycle continues and centromeric cohesins are lost, allowing the chromosomes to segregate during meiosis II (top right panel). Thus, *mam1*Δ mutants delay in metaphase I for 1–2 hr, the time it takes cells to progress from metaphase I to metaphase II. This delay is bypassed in a *spo11*Δ *mam1*Δ *pREC8-SCC1* mutant (bottom) in which centromeric cohesins are lost at the onset of anaphase I (bottom middle panel). During meiosis II, sister chromatids segregate in a random manner because of the lack of centromeric cohesins (bottom right panel).

(E) *spo11*Δ *mam1*Δ (closed squares, A6838), *spo11*Δ *mam1*Δ *pREC8-SCC1-3HA* (open triangle, A11064), and *spo11*Δ *mam1*Δ *spo13*Δ (closed circles, A11099) were elutriated and sporulated as described in the Experimental Procedures. The percentage of cells with two or more nuclei (first panel), metaphase I spindles (second panel), anaphase I spindles (third panel), and the sum of metaphase I, anaphase I, or metaphase II spindles (fourth panel) was determined at the indicated time points.

equational divisions simultaneously (Figure 2A). This is in contrast to FEAR network mutants, in which Rec8 is lost from the chromosomes in a stepwise manner, first along chromosome arms during meiosis I, then around centromeric regions in meiosis II [29, 30]. Moreover, deletion of *SPO11*, which eliminates recombination and leads to the complete loss of equationally segregating chromosomes in *spo12*Δ mutants (Figure 1B; [29]). In contrast to *spo12*Δ, *spo13*Δ cells segregated chromosome V exclusively in an equational manner when *SPO11* was deleted (Figure 1C). These results indicate that the generation of *spo13*Δ dyads with equationally segregated chromosomes is not due to an uncoupling of meiotic events, as observed in FEAR network mutants.

Spo13 Maintains Centromeric Cohesion during Meiosis I

Spo13 has been implicated in regulating the stepwise loss of cohesins from chromosomes during meiosis. Little or no Rec8 is detected around centromeres in binucleate *spo13*Δ cells [8]. Furthermore, overexpression of *SPO13* in mitotic cells prevents cleavage of the cohesin subunits, Scc1 and Mcd1, or ectopically expressed Rec8 [25]. To examine in detail when Rec8 is lost from chromosomes in *spo13*Δ cells progressing through meiosis, we examined the localization of Rec8 by chromosome spreads. Consistent with previous reports, in wild-type cells the percentage of cells that had lost Rec8 from chromosome arms paralleled the completion of meiosis I, and the percentage of cells that had no detectable Rec8 staining paralleled those that had completed meiosis II (Figure 2A, left; [8, 9]). This stepwise loss of Rec8 was reflected in the 1 hr difference between the appearance of cells that had lost Rec8 from chromosome arms and that of cells that had lost all Rec8 staining (Figure 2A, left; Figure S1 in the Supplemental Data available with this article online). In contrast to wild-type cells, Rec8 was lost from chromosome arms and centromeres at essentially the same time in *spo13*Δ cells (Figure 2A, right). The slight difference in the kinetics with which arm cohesion and all cohesion is lost is due to some *spo13*Δ cells containing a small but detectable pool of Rec8 associated with centromeric regions (an example is shown in Figure 2C, middle panel; [8]).

To investigate whether this residual pool of Rec8 could maintain cohesion between sister chromatids, we performed a functional assay for centromeric cohesion activity. Because *spo13*Δ cells undergo only a single meiotic division, it was necessary to use an assay that probes for centromeric cohesion activity during meiosis I rather than the traditional method of assessing sister chromatid segregation behavior in meiosis II. This meiosis I assay takes advantage of the observation that *mam1*Δ mutants delay chromosome segregation until meiosis II (Figure 2D; [19]). *MAM1* is a meiosis-specific gene that is required for sister kinetochore coorientation. When *MAM1* is deleted, sister kinetochores are bioriented in meiosis I. The segregation of bioriented chromosomes, as in mitosis, requires the complete removal of cohesin. The loss of cohesin in *mam1*Δ cells, however, is stepwise. Thus, centromeric Rec8 prevents chromosome segregation in meiosis I in *mam1*Δ cells until it is destroyed in the second round of Rec8 cleavage, at the time when wild-type cells are undergoing meiosis II (Figure 2D; [17, 19]). This delay can be bypassed if centromeric cohesion is lost in meiosis I, such as when Rec8 is replaced with its mitotic homolog Scc1/Mcd1 (Figures 2D and 2E; [19, 20]). Because Scc1/Mcd1 cannot support meiotic recombination resulting in a prophase arrest, it was necessary to conduct the assay under conditions that eliminated recombination by the deletion of *SPO11* [19]. Inactivation of *SPO11* did not affect the pattern of Rec8 loss in either wild-type or *spo13*Δ cells, indicating that abolishing recombination would not interfere with the assay (Figures 2B and S1).

Deletion of *SPO13* abolished the delay in chromosome segregation of *mam1*Δ cells to the same extent as replacement of *REC8* with *SCC1/MCD1*, most notably around the 4 hr time point, when most of the cells are in meiosis I (Figure 2E, top left). The rescue of the delay was more apparent when meiotic spindle morphology was examined. *mam1*Δ mutants were delayed in metaphase I as a result of centromeric cohesins preventing chromosome segregation of bioriented sister chromatids. Deletion of *SPO13* or expression of *SCC1/MCD1* in place of *REC8* abolished the delay to a similar extent, as evidenced by the disappearance of cells with metaphase I spindles and the accumulation of cells with anaphase I spindles (Figure 2E, top right and bottom left). Furthermore, the sum of cells with metaphase I, anaphase I, prophase II, and metaphase II spindles was the same in all three strains (Figure 2E, bottom right), suggesting that the metaphase I delay in *mam1*Δ mutants is due to centromeric cohesins, which are removed in meiosis II, rather than some defect in metaphase I spindle function. Our results suggest that *spo13*Δ cells lose centromeric cohesins prematurely and that the residual Rec8 detected around centromeres in some *spo13*Δ cells is insufficient for maintaining sister chromatid cohesion.

Spo13 Acts Independently of Sgo1 in Protecting Centromeric Cohesins

Recently, *SGO1* was identified as being required for preventing the loss of Rec8 from centromeric regions during meiosis I [15–18]. Sgo1 localizes to kinetochores from prophase I to metaphase II during the meiotic cell cycle (Figure 3C; [16, 17]). To determine whether *SPO13* protected Rec8 from being removed from centromeric regions by regulating Sgo1, we first examined the localization of Sgo1 in cells lacking *SPO13*. Sgo1 localized to kinetochores in *spo13*Δ mutants with kinetics that were indistinguishable from those of wild-type cells (Figure 3A, bottom right; Figure 3C), although the Sgo1 foci often appeared smaller. Furthermore, we did not observe any differences in Sgo1 protein levels or posttranslational modifications between wild-type and *spo13*Δ cells, as judged from Western blot analysis (Figure 3B). Note that the 1 hr delay in the accumulation and degradation of Sgo1 in the *spo13*Δ mutant is due to *spo13*Δ cells entering meiosis later than wild-type cells in this particular experiment (see Figure 3A).

To test whether *SPO13* requires *SGO1* to prevent cohesin removal, we examined whether the mitotic

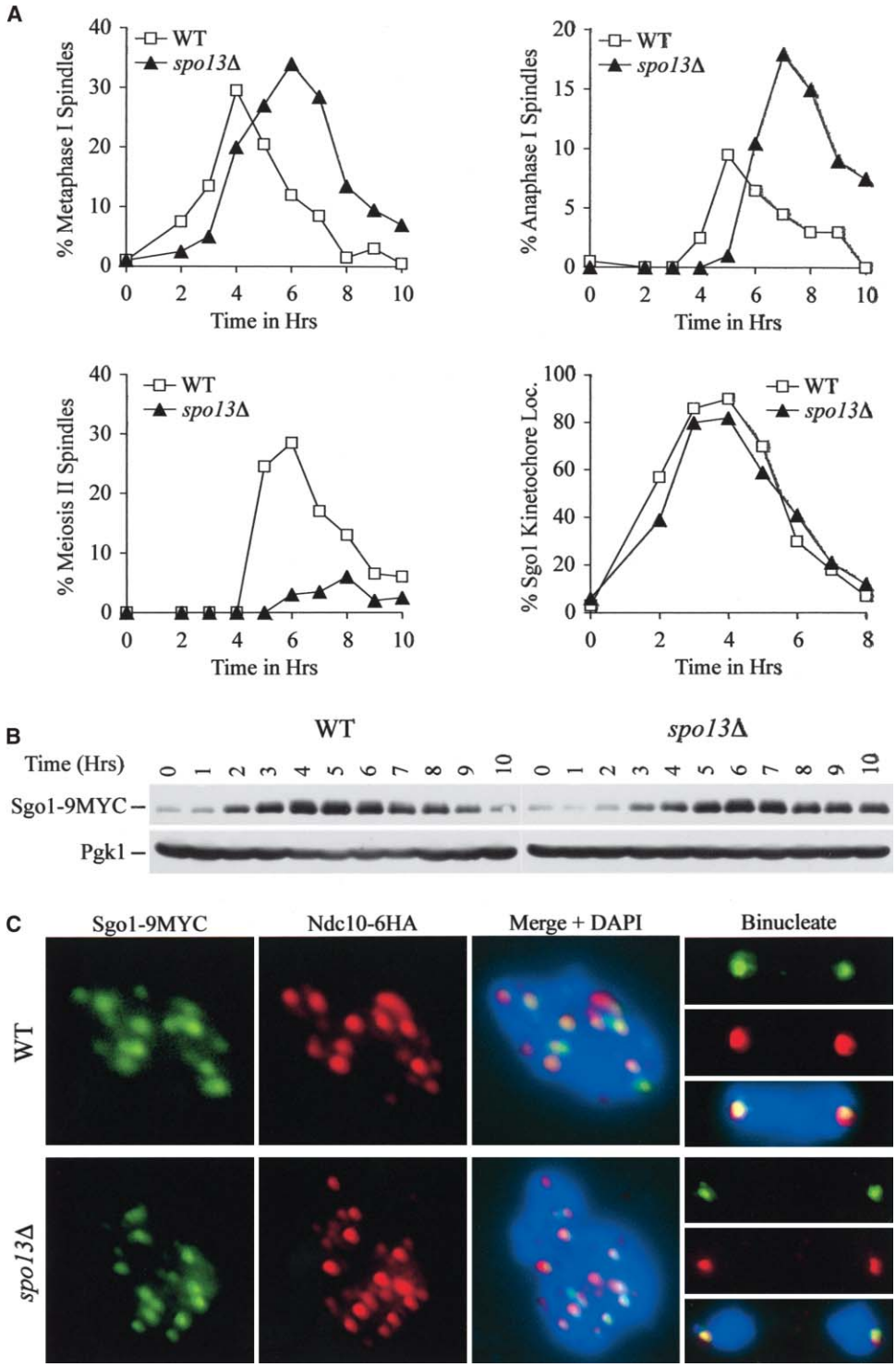
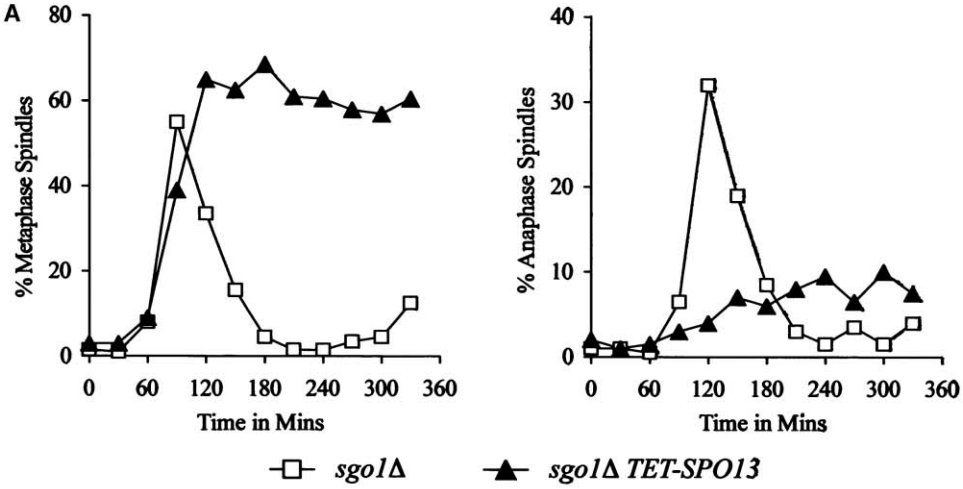


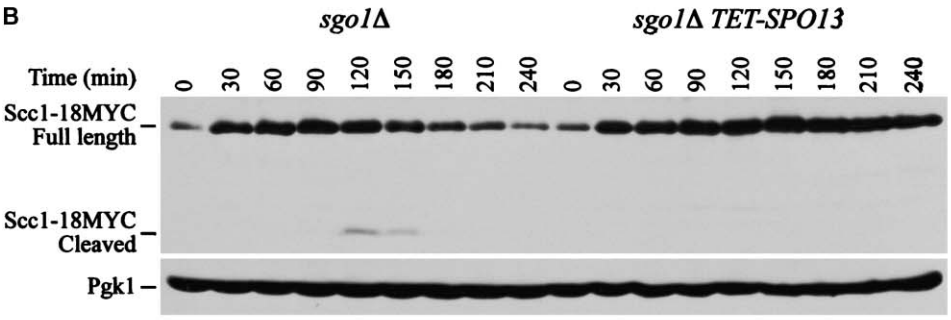
Figure 3. *SPO13* Does Not Regulate Sgo1 Localization or Protein Levels

(A) Wild-type (open squares, A10461) and *spo13*Δ (closed triangles, A10755) cells carrying *SGO1-9MYC* and *NDC10-6HA* fusions were sporulated as described. The percentage of cells with metaphase I spindles (top left), anaphase I spindles (top right), and meiosis II spindles (bottom left) and the percentage of Sgo1 colocalizing with Ndc10 on chromosome spreads (bottom right) was determined at the indicated time points.

(B) Western blot samples from (A) were used for monitoring Sgo1 protein levels during meiosis. Pgk1 is shown as a loading control. The 1 hr delay in the accumulation and degradation of Sgo1 in *spo13*Δ cells is due to a delay of the *spo13*Δ strain in entering meiosis in this experiment (see meiotic cell cycle progression in [A]).

(C) Examples of Sgo1 localization on meiotic spreads from wild-type (top) and *spo13*Δ (bottom) taken 5 hr after induction of sporulation. Sgo1-9MYC is shown in green, Ndc10-6HA in red, and DAPI in blue. The first three panels show Sgo1 staining in mononucleated cells; the last panel shows a binucleated cell.





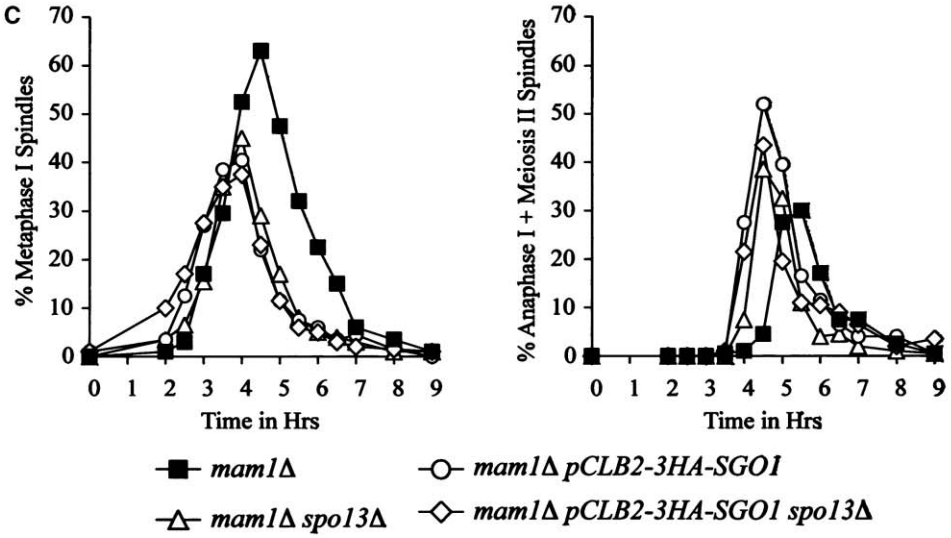


Figure 4. Relationship between Spo13 and Sgo1

(A and B) *sgo1*Δ (open squares, A11032) and *sgo1*Δ *TET- SPO13* (closed triangles, A11033) cells carrying a *SCC1-18MYC* fusion were grown at room temperature in YEPR containing doxycycline (5 μg/ml) to inhibit *SPO13* expression. Cells were then washed to remove the doxycycline and arrested in G1 with α-factor (5 μg/ml) for 4 hr, followed by release into YEPR lacking pheromone and doxycycline at room temperature. After 90 min, when more than 90% of cells were budded, 5 μg/ml α-factor was added to prevent entry into the next cell cycle. The percentage of cells with metaphase spindles ([A], left graph) or anaphase spindles ([A], right graph) was determined at the indicated time points. The total amount of Scc1-18MYC and Pgk1 was determined by Western blot analysis (B).

(C) *mam1*Δ (closed squares, A6958), *mam1*Δ *pCLB2-3HA-SGO1* (open circles, A11249), *mam1*Δ *spo13*Δ (open triangles, A7027), and *mam1*Δ *pCLB2-3HA-SGO1 spo13*Δ (open diamonds, A11250) cells were elutriated and sporulated as described. The percentage of cells with metaphase I spindles (left) and the sum of anaphase I and meiosis II spindles (right) were determined at the indicated time points.

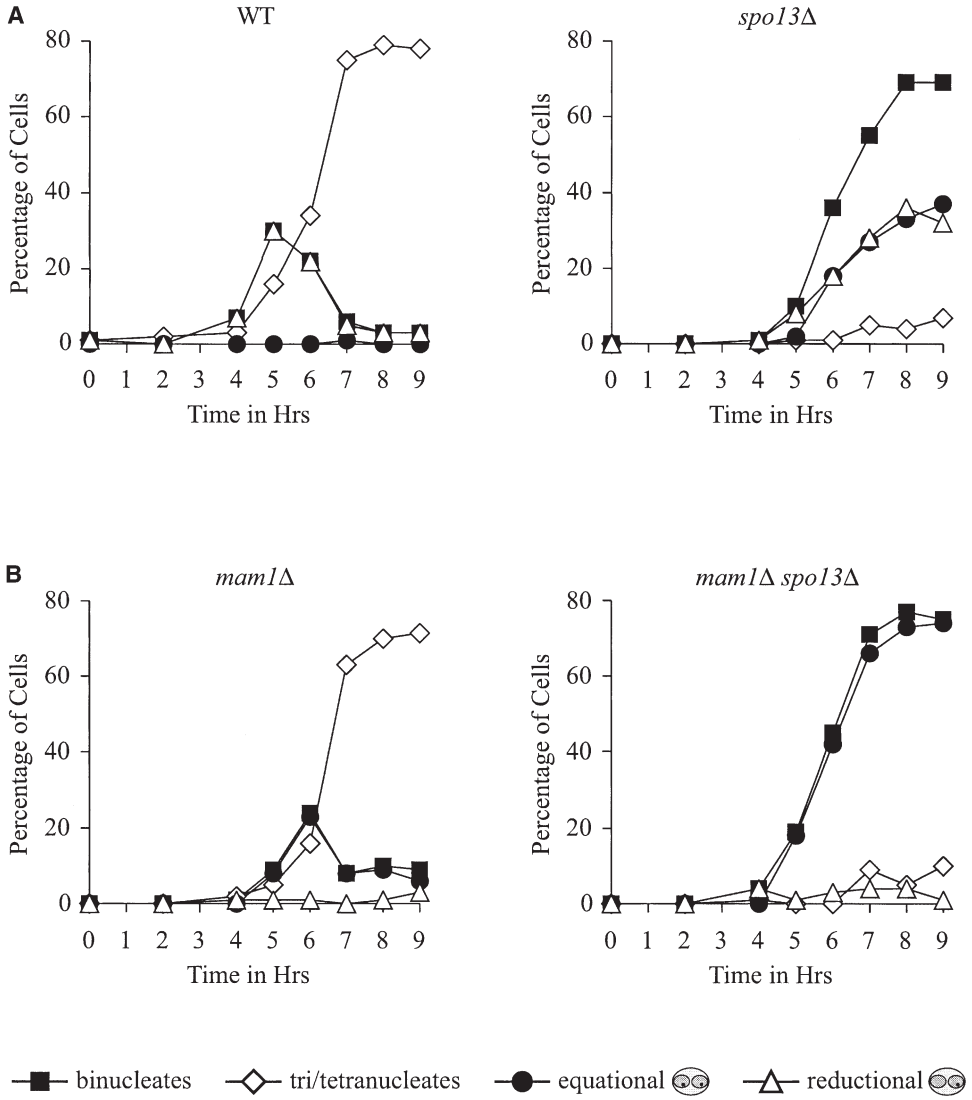


Figure 5. The Reductional Chromosome Segregation in *spo13*Δ cells Depends on *MAM1*

Wild-type ([A] left, A5811), *spo13*Δ ([A] right, A5891), *mam1*Δ ([B] left, A7315), and *mam1*Δ *spo13*Δ ([B] right, A7497) cells carrying heterozygous CENV GFP dots were sporulated. The percentage of binucleated (closed squares) and tri/tetranucleated cells (open diamonds) as well as binucleated cells with GFP label in one of the two nuclei (open triangles; reductional) and binucleated cells with GFP label in both nuclei (closed circles; equational) was determined at the indicated time points. Note that the experiments shown in Figures 1 and 5 were carried out at the same time; hence, the graphs for wild-type and *spo13*Δ cells are the same in both figures.

metaphase arrest brought about by high levels of Spo13 required *SGO1*. Overexpression of *SPO13* was shown to prevent the cleavage of Scc1/Mcd1 and Rec8 by separase in mitosis and to thus result in a metaphase arrest [25, 26, 33]. Deletion of *SGO1* did not bypass this metaphase arrest (Figure 4A), nor did it allow cleavage of Scc1/Mcd1 (Figure 4B). This result indicates that Spo13 can inhibit cohesin cleavage in an *SGO1*-independent manner.

The finding that Spo13 can prevent cleavage of Scc1/Mcd1 in the absence of *SGO1* and that *SPO13* was not required for Sgo1 localization to chromosomes during meiosis raised the possibility that the two proteins function in parallel but not redundant pathways to protect Rec8 from being removed from centromeres during meiosis I. To test this hypothesis, we examined the effects of deleting both genes on centromeric cohesion by employing the functional centromeric cohesion assay and using *mam1*Δ cells as described above. *sgo1*Δ mutants exhibit severe defects during the mitotic divisions, resulting in a high frequency of mitotic chromosome nondisjunction and a delay in progression through meiosis, making a comparison with *spo13*Δ cells difficult [15–17]. To circumvent this problem, we created a meiosis-specific depletion allele of *SGO1* by placing *SGO1* under the mitosis-specific *CLB2* promoter (*pCLB2-3HA-SGO1*; [32]). *pCLB2-3HA-SGO1* cells exhibited no obvious mitotic defects but, similar to *sgo1*Δ mutants, segregated chromosomes randomly during meiosis II, indicative of premature loss of centromeric cohesion during meiosis

I (A. Marston, personal communication). Deletion of *SPO13* or depletion of Sgo1 completely rescued the metaphase I delay of *mam1*Δ cells, suggesting that both are essential for the retention of centromeric cohesion (Figure 4C). The double mutant exhibited the same phenotype as either single mutant in this assay (Figure 4C). However, because each single mutant is completely defective in the retention of centromeric cohesion, any additive effects would not be apparent. Taken together, our results suggest that *SPO13* either functions in parallel to or downstream of *SGO1*.

SPO13 Is Partially Defective in Sister Kinetochore Coorientation

Sister kinetochore coorientation is established and maintained by a complex consisting of at least three subunits: Mam1, Lrs4, and Csm1 [19, 20]. The finding that *spo13*Δ mutants segregate chromosomes reductionally and equationally with equal frequency (Figures 1C and 5A) simultaneously suggests that *spo13*Δ mutants lose centromeric cohesion prematurely and that sister kinetochore coorientation is aberrant. The mixed segregation phenotype could be due to sister kinetochores attaching randomly so that there is an equal frequency of bipolar and monopolar attachment. Alternatively, kinetochore coorientation could be partially defective in *spo13*Δ mutants. To distinguish between these possibilities, we examined whether the reductional segregation in *spo13*Δ mutants depends on *MAM1*. If sister kinetochores were attaching to microtubules in a random fashion, inactivation of *MAM1* should not affect the segregation pattern observed in *spo13*Δ mutants. However, *spo13*Δ *mam1*Δ double mutants segregated chromosomes in an exclusively equational manner (Figure 5B), indicating that the reductional segregation observed in *spo13*Δ mutants depends on monopolin complex function. This finding suggests that sister kinetochores do not attach to microtubules randomly in *spo13*Δ mutants and that the monopolin complex is able to generate coorientation of some sister kinetochores in *spo13*Δ cells. A certain fraction of sister kinetochores, however, fails to coorient in *spo13*Δ mutants, indicating that some aspect of coorientation is impaired in this mutant.

SPO13 Is Required for the Maintenance of Monopolins at Kinetochores

The partial defect observed in kinetochore coorientation in *spo13*Δ mutants prompted us to determine whether Spo13 regulates the localization of the monopolin complex. In wild-type cells, Mam1 localizes to the nucleus and to kinetochores, as determined by colocalization with the kinetochore component Ndc10 in late prophase I and metaphase I (Figure 6A, left; [19]). In *spo13*Δ mutants, the localization of Mam1 to the nucleus and the level of Mam1 protein were not affected (Figure 6A, right; Figure 6C). However, the association of Mam1 with kinetochores was. Mam1 appeared to transiently localize to kinetochores in at least a fraction of the cells during prophase I but was absent from kinetochores during metaphase I (Figure 6A, right). Localization of Lrs4, a second component of the monopolin complex, showed similar abnormalities in *spo13*Δ mutants (Figures 7A and 7B). In wild-type cells, Lrs4 resides in the nucleolus but associates with kinetochores during late prophase I and metaphase I (Figures 7A and 7B; [20]). In *spo13*Δ cells, Lrs4 associated with kinetochores in a fraction of cells during late prophase I but was absent from kinetochores during metaphase I (Figures 7A and 7B). Similar results were obtained in *spo11*Δ *spo13*Δ mutants, in which all chromosomes segregate equationally (Figure S2).

To examine in more detail whether Lrs4 was capable of associating with kinetochores in *spo13*Δ mutants, we examined the binding of the protein to kinetochores by using chromatin immunoprecipitation (ChIP) during early prophase I (4 hr after induction of meiosis) when the proteins appeared to be associated with kinetochores, as evidenced by immunolocalization studies. Lrs4 was detected at centromeres but not on chromosome arms in *spo13*Δ mutants, almost to the same extent as in wild-type cells during early prophase I (Figures 8A and 8B). At later time points, Lrs4 association with kinetochores was lost, as evidenced by ChIP in *spo13*Δ cells (Figure S3) and chromosome spreads (Figures 7A and 7B). These results suggest that *SPO13* is required for maintaining the monopolin complex at kinetochores.

A Fraction of Spo13 Localizes to Kinetochores

Spo13's role in maintaining centromeric cohesion and kinetochore coorientation during meiosis I prompted us to investigate whether Spo13 itself was found at kinetochores. Spo13 was found enriched at the majority of kinetochores, as evidenced by the colocalization of some Spo13 foci with the kinetochore component Ndc10 (Figure 9). ChiP showed that Spo13 was also enriched at centromeric regions of chromosome VI (Katis et al., pages 2183–2196 of this issue [34]). However, it is important to note that Spo13 was also found to be weakly associated with other regions of the genome, indicating that a fraction but not all of Spo13 associates with centromeric regions.

Discussion

Our results define Spo13 as a key regulator of meiotic chromosome segregation. It is required for two unique aspects of meiosis I, namely the organization of sister kinetochore coorientation and the protection of centromeric cohesion. In the absence of *SPO13*, cohesins around centromeres are lost prematurely during meiosis I. Spo13 also promotes sister kinetochore coorientation by maintaining the localization of the monopolin complex at kinetochores. In the absence of *SPO13*, Mam1 and Lrs4 disassociate from kinetochores prematurely during pro-metaphase I and metaphase I, resulting in a partial defect in sister kinetochore coorientation in *spo13*Δ cells.

The Role of Spo13 and Sgo1 in Regulating Centromeric Cohesion

Our data together with previously published results suggest that *SPO13* is required for maintaining cohesins around centromeres during meiosis I, probably by interfering with Rec8 cleavage (Figure 4; [8, 25, 26]). Spo13, however, does not act alone to protect cohesins

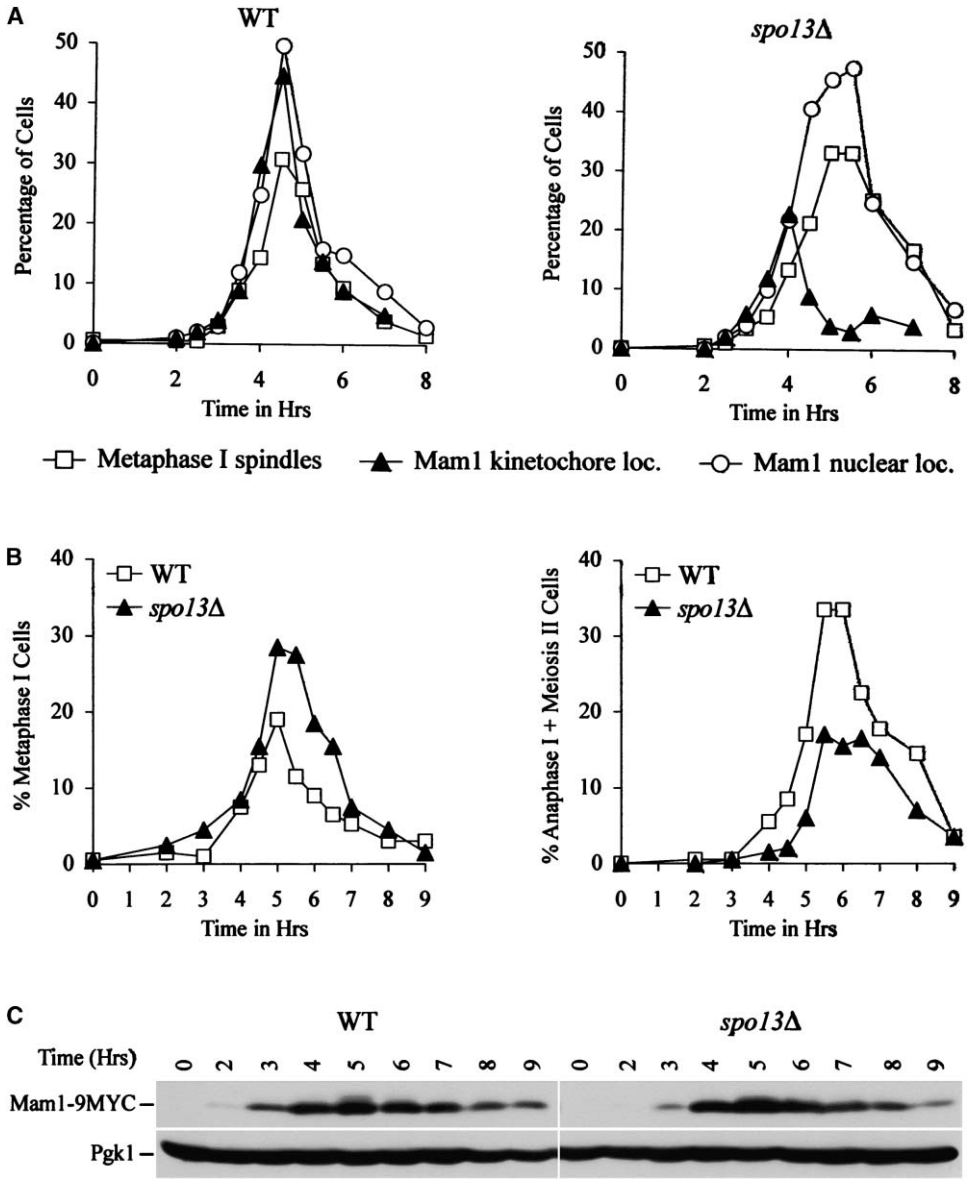


Figure 6. SPO13 Is Required for Mam1 Localization at Kinetochores

(A) Wild-type (left, A7097) and *spo13*Δ (right, A7451) cells carrying *MAM1-9MYC* and *NDC10-6HA* fusions were elutriated and sporulated as described in the Experimental Procedures. The percentage of cells with metaphase I spindles (open squares), Mam1 localized to the nucleus (open circles), and Mam1 colocalized with Ndc10 on chromosome spreads (closed triangles) was determined at the indicated time points.

(B and C) Wild-type (A7097) and *spo13*Δ (A7451) carrying *MAM1-9MYC* and *NDC10-6HA* fusions were sporulated, and the percentage of cells with metaphase I spindles ([B] left) and the sum of anaphase I and meiosis II spindles (B right) were determined at the indicated time points.

(C) Protein levels of Mam1-9MYC and Pgk1 were monitored by Western blot analysis.

around centromeres. A MEI-S332 family member known as Sgo1 has recently been shown to be essential for preventing cohesin loss from centromeric regions during meiosis I in budding yeast (Figure 4; [15–17]). Our analysis of the relationship between *SPO13* and *SGO1* revealed that *SPO13* is not required for Sgo1 localization at kinetochores and that *SGO1* is not required for Spo13 to inhibit anaphase onset during mitosis when Spo13 is overexpressed. These findings formally place *SPO13* downstream of or in parallel to *SGO1*. The finding that *spo13*Δ *sgo1*Δ mutants exhibit the same phenotype as either single mutant with respect to premature loss of Rec8 from centromeric regions would suggest that the two genes function in the same pathway. However, given that either single mutant exhibits complete loss of centromeric cohesion function during meiosis I, any enhancement in the double mutant is unlikely to be apparent. We therefore cannot exclude the possibility that Spo13 functions in a parallel but not redundant pathway to Sgo1.

Importantly, our results also show that Sgo1 localization to kinetochores alone is not sufficient for protecting Rec8 from removal around centromeres during meiosis I. In the absence of *SPO13*, Sgo1 is at kinetochores,

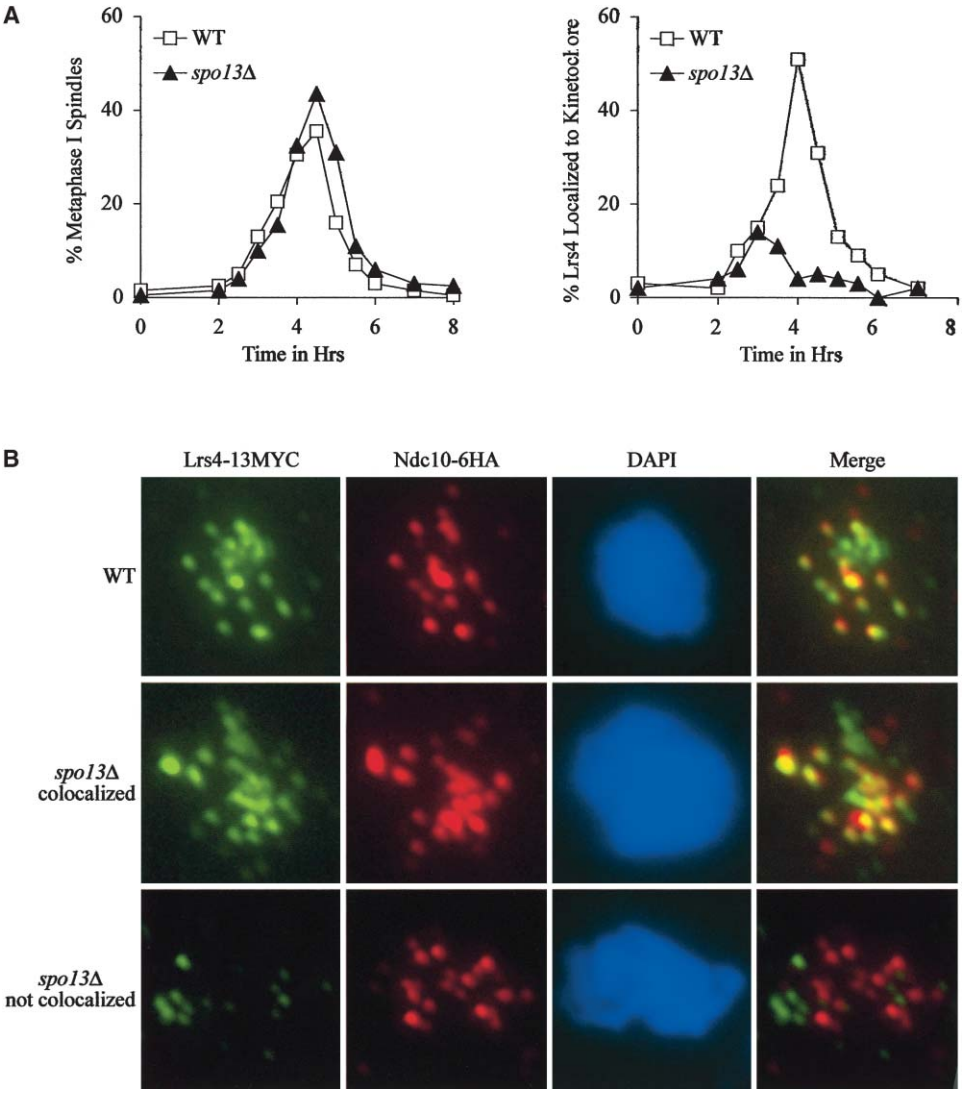


Figure 7. *SPO13* Is Required for Lrs4 to Be Maintained at Kinetochores

(A) Wild-type (open squares, A9043) and *spo13*Δ (closed triangles, A9045) cells carrying *LRS4-13MYC* and *NDC10-6HA* fusions were elutriated and sporulated as described in the Experimental Procedures. The percentage of cells with metaphase I spindles (left) and Lrs4 colocalized with Ndc10 on chromosome spreads (right) was determined at the indicated time points.

(B) Examples of Lrs4 localization in wild-type (top) and *spo13*Δ (middle and bottom) cells taken 4 hr after induction of sporulation. Lrs4-13MYC is shown in green (first panel), Ndc10-6HA in red (second panel), and DAPI in blue (third panel). A merge of Ndc10-6HA and Lrs4-13Myc is shown in the fourth panel. The middle row shows an example of a *spo13*Δ cell in where Lrs4 is colocalized with Ndc10, and the bottom is an example of a *spo13*Δ cell where Lrs4 and Ndc10 do not colocalize.

yet Rec8 is removed prematurely. We speculate that protection of centromeric cohesins by the combined activity of both Spo13 and Sgo1 allows for spatial and developmental specificity. Sgo1, which is present at kinetochores during both mitosis and meiosis, provides spatial specificity for centromeric regions. Spo13 and Rec8, whose localization is not restricted to centromeric regions but which are present only during meiosis, provide developmental specificity; that is, protection occurs only during meiosis. The fact that Spo13 is degraded during anaphase I (data not shown; [35]) would further restrict protection to meiosis I.

The Role of Spo13 in Kinetochore Coorientation

Sister kinetochore coorientation is established and maintained by a complex consisting of at least three subunits: Mam1, Lrs4, and Csm1 [19, 20]. This complex localizes to kinetochores during prophase and metaphase I and dissociates from these structures at the onset of anaphase I (Figures 6–8; [19, 20]). The localization of this complex to kinetochores is regulated by the polo-like kinase Cdc5 [32, 36, 37] and by Spo13 (this study). Cdc5 is likely to be required for the establishment of coorientation because Cdc5 depletion mutants are completely defective in kinetochore coorientation and Mam1 does not appear to localize to kinetochores in prophase I of Cdc5-depleted cells [32, 37]. In contrast, Mam1 and Lrs4 appear to transiently associate with kinetochores during prophase I but are absent during metaphase I in the *spo13*Δ mutant. This is true even in *spo13*Δ *spo11*Δ mutants in which all chromosomes segregate equationally. We cannot exclude the possibil-

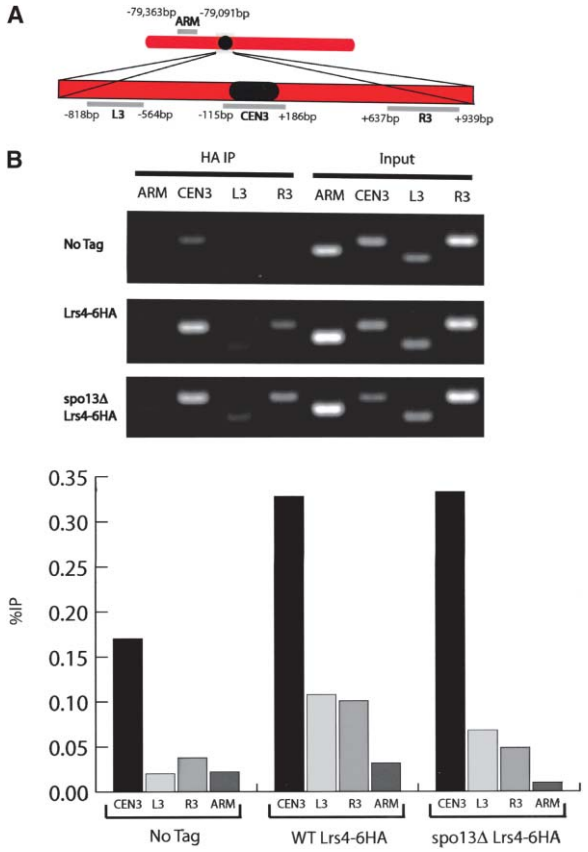


Figure 8. Spo13 Is Required for Lrs4 to Be Maintained at Centromere Chromatin

(A) Diagram of chromosome III primer sets with respect to the chromosome III centromere used for chromatin immunoprecipitation analysis in (B).

(B) PCR analysis of total DNA (input) and sequences immunoprecipitated with antibodies against the HA epitope. Anti-HA antibodies precipitated pericentric sequences similarly to α-Cep3 antibody (data not shown) in a wild-type strain expressing Lrs4-6HA (A11016) and to a similar extent in a *spo13*Δ strain expressing the same tagged version of Lrs4 (A11018) but not in an untagged strain (A4962). Samples were harvested 4 hr after transfer into sporulation medium. Similar results were also obtained for centromeres of chromosome IV and VI (data not shown).

ity that *SPO13* also plays a role in the initial association of the monopolin complex with kinetochores, but our localization and chromatin immunoprecipitation studies on Mam1 and Lrs4 clearly show that Spo13 is required for the maintenance of coorientation. Despite the differences in Mam1 localization in Cdc5-depleted cells and *spo13*Δ cells, it is possible that Cdc5 and Spo13 cooperate to regulate monopolin complex function. Spo13 and Cdc5 interact in vivo and in a two-hybrid assay (B.H.L. and S.P., unpublished observation). Perhaps Spo13 is important for Cdc5 to continually promote the localization of the monopolin complex to kinetochores throughout pro-metaphase I and metaphase I, before stable attachment is established. Consistent with this idea is the observation that a fraction of Spo13 is found at kinetochores during prophase I and metaphase I.

The Relationship between Recombination and Spo13 in Sister Kinetochore Coorientation

Two decades ago Esposito and coworkers made the interesting observation that the *spo13*Δ mutant can restore spore viability to mutants defective in the initial steps of recombination [38, 39]. It became apparent that this was due to an essentially mitosis-like division occurring in cells lacking both *SPO13* and recombination genes. Furthermore, it was discovered that recombination can partially substitute for *SPO13* function and that recombination-deficient mutants eliminate all reductional chromosome segregations in *SPO13* mutants, leading to an entirely equational division [24]. How can we reconcile this observation with the facts that Spo11 does not play a role in kinetochore coorientation (Figure 1B; [8]) and that Mam1 localization is similar in *spo13*Δ single and *spo11*Δ *spo13*Δ double mutants? We speculate that during chromosome attachment in pro-metaphase I, some bivalents (pairs of homologous chromosomes connected through at least one chiasma) attach to the meiosis I spindle correctly, in that the kinetochores of the homologs bind microtubules emanating from opposite spindle poles. Other bivalents attach to the meiosis I spindle incorrectly, in that kinetochores of the homologs bind microtubules emanating from the same spindle pole. These incorrect attachments will be severed to prevent homolog nondisjunction. Because *spo13*Δ mutants fail to maintain the monopolin complex

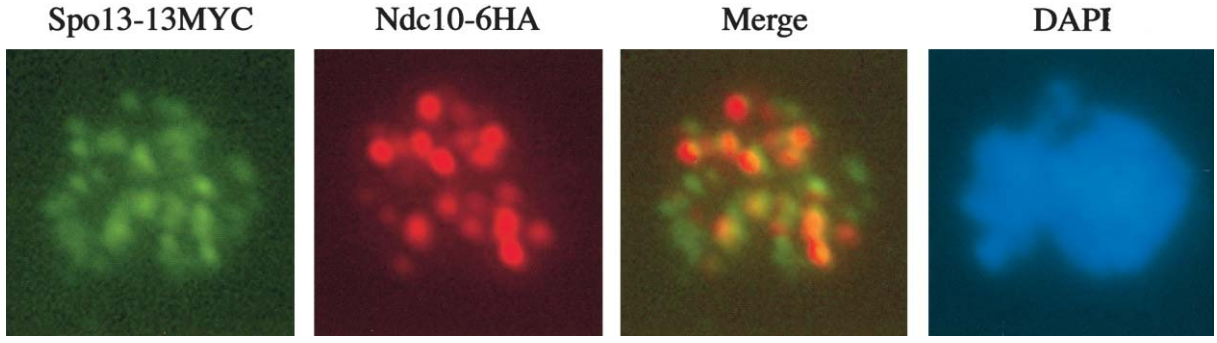


Figure 9. A Fraction of Spo13 Colocalizes with the Kinetochore Component Ndc10

Wild-type cells carrying *SPO13-13MYC* and *NDC10-6HA* fusions (A9452) were sporulated and spread to examine Spo13 and Ndc10 localization. Spo13-13MYC is shown in green (first panel), Ndc10-6HA in red (second panel), and DNA in blue (fourth panel). A merge of Ndc10-6HA and Lrs4-13Myc is shown in the third panel.

Table 1. Strains

Strain Number	Relevant Genotype
A5811	MATa/α *LEU2::pURA3-TetR-GFP/+ CENV::TetOx224/+*
A5814	MATa/α *LEU2::pURA3-TetR-GFP/+ CENV::TetOx224/+ spo12Δ/spo12Δ*
A7314	MATa/α *LEU2::pURA3-TetR-GFP/+ CENV::TetOx224/+ spo11Δ/spo11Δ*
A9168	MATa/α *LEU2::pURA3-TetR-GFP/+ CENV::TetOx224/+ spo11Δ/spo11Δ spo12Δ/spo12Δ*
A5891	MATa/α *LEU2::pURA3-TetR-GFP/+ CENV::TetOx224/+ spo13Δ/spo13Δ*
A7170	MATa/α *LEU2::pURA3-TetR-GFP/+ CENV::TetOx224/+ spo11Δ/spo11Δ spo13Δ/spo13Δ*
A4758	MATa/α *NDC10-13MYC/+ REC8-3HA/+*
A4837	MATa/α *NDC10-13MYC/+ REC8-3HA/+ spo13Δ/spo13Δ*
A7240	MATa/α *NDC10-13MYC/+ REC8-3HA/+ spo11Δ/spo11Δ*
A7810	MATa/α *NDC10-13MYC/+ REC8-3HA/+ spo11Δ/spo11Δ spo13Δ/spo13Δ*
A6838	MATa/α *spo11Δ/spo11Δ mam1Δ/mam1Δ*
A11064	MATa/α *spo11Δ/spo11Δ rec8Δ/rec8Δ pREC8::pREC8-SCC1-3HA/ pREC8::pREC8-SCC1-3HA mam1Δ/mam1Δ*
A11099	MATa/α *spo11Δ/spo11Δ mam1Δ/mam1Δ spo13Δ/spo13Δ*
A10461	MATa/α *SGO1-9MYC/SGO1-9MYC NDC10-6HA/NDC10-6HA*
A10755	MATa/α *SGO1-9MYC/SGO1-9MYC NDC10-6HA/NDC10-6HA spo13Δ/spo13Δ*
A11032	W303 MATa *sgo1Δ SCC1-18MYC*
A11033	W303 MATa *sgo1Δ SCC1-18MYC TET-SPO13*
A6958	MATa/α *mam1Δ/mam1Δ*
A7027	MATa/α *mam1Δ/mam1Δ spo13Δ/spo13Δ*
A11249	MATa/α *mam1Δ/mam1Δ pCLB2-3HA-SGO1/pCLB2-3HA-SGO1*
A11250	MATa/α *mam1Δ/mam1Δ pCLB2-3HA-SGO1/pCLB2-3HA-SGO1 spo13Δ/spo13Δ*
A7315	MATa/α *LEU2::pURA3-TetR-GFP/+ CENV::TetOx224/+ mam1Δ/mam1Δ*
A7497	MATa/α *LEU2::pURA3-TetR-GFP/+ CENV::TetOx224/+ mam1Δ/mam1Δ spo13Δ/spo13Δ*
A7097	MATa/α *MAM1-9MYC/MAM1-9MYC NDC10-6HA/NDC10-6HA*
A7451	MATa/α *MAM1-9MYC/MAM1-9MYC NDC10-6HA/NDC10-6HA spo13Δ/spo13Δ*
A9043	MATa/α *LRS4-13MYC/LRS4-13MYC NDC10-6HA/NDC10-6HA*
A9045	MATa/α *LRS4-13MYC/LRS4-13MYC NDC10-6HA/NDC10-6HA spo13Δ/spo13Δ*
A4962	MATa/α
A11016	MATa/α *MAM1-9MYC/+ LRS4-6HA/+*
A11018	MATa/α *MAM1-9MYC/+ LRS4-6HA/+ spo13Δ/spo13Δ*

at kinetochores, chromosomes that have detached must now reattach in a bipolar manner as in mitosis as a result of the absence of a monopolin complex to satisfy the tension checkpoint [40]. The differences in the percentage of equational segregation between different chromosomes and between different strain backgrounds can then be explained by a difference in the tendencies of particular chromosomes to acheive the correct initial attachment. For example, chromosome V in SK1 might acheive correct initial attachment half of the time, explaining why 50% of chromosomes V in *spo13Δ* mutants segregate equationally and 50% segregate reductionally (Figures 1C and 5A).

In this model, the shift to an entirely equational segregation pattern in *spo13Δ spo11Δ* mutants can be explained as follows. Initially, kinetochores are cooriented in *spo13Δ spo11Δ* mutants but, due to the absence of a physical linkage between the homologs, tension is absent. The lack of tension at the kinetochores is recognized by the cell, kinetochore microtubule attachments are severed, and the spindle checkpoint is activated. During this process, monopolin complexes dissociate from kinetochores as a result of the absence of *SPO13*, and all chromosomes must reattach in a bipolar manner to satisfy the spindle checkpoint in *spo11Δ spo13Δ* mutants. Consistent with this model is the observation that, in *spo11Δ spo13Δ* double mutants, deletion of the spindle checkpoint component *MAD2* leads to a mixed segregation pattern similar to that of *spo13Δ* single mutants (B.H.L., unpublished observations; [26]).

Spo13: A Meiosis I Clock?

Spo13 regulates two major aspects of meiosis I chromosome segregation, maintenance of centromeric cohesion and maintenance of kinetochore coorientation. In the absence of *SPO13*, both cohesin and monopolin complexes are prematurely lost from kinetochores, resulting in meiosis II- and meiosis I-like chromosome segregation patterns occurring at the same time. These observations, along with the timely degradation of Spo13 at the end of meiosis I, raise the intriguing possibility that Spo13 acts as a molecular timer for meiosis I. The presence of Spo13 prevents meiosis II events such as kinetochore biorientation and loss of centromeric cohesin from taking place until it is degraded in anaphase I, thus temporally separating meiosis I from meiosis II.

Experimental Procedures

Strains used in this study are described in Table 1 and were derivatives of SK1, unless otherwise noted.

Constructs

The *pCLB2-3HA-SGO1* strain was constructed by a one-step PCR-based gene replacement method [41] with plasmid *pFA6a-pCLB2-3HA-KanMX6* as the template [32]. *NDC10-13MYC, LRS4-13MYC, LRS4-6HA*, and *mam1::TRP1* were constructed by a one-step PCR-based gene replacement method [41]. *REC8-3HA, spo13::hisG,* and *spo11::URA3* were described in [8]. *MAM1-9MYC, NDC10-6HA, pREC8-SCC1-3HA*, *spo11::TRP1*, and *rec8::KanMX* as well as the CENV GFP dots were described in [19]. *spo12::LEU2* was described in [29]. *SGO1-9MYC* and *sgo1::KanMX6* were described in [16]. *TET-SPO13* and *SCC1-18MYC* were described in [25].

Sporulation Condition

Cells were grown to saturation in YPD (YEP + 2% glucose) for 24 hr, diluted into YPA (YEP + 2% KAc) at OD_{600} = 0.2, and grown overnight. The cells were then washed with sterilized water and resuspended in SPO medium (0.3% KAc [pH = 7.0]) at OD_{600} = 1.8

to induce sporulation. Cells were grown at 30°C unless otherwise noted.

Elutriation

Cells were grown to saturation in YPD (YEP + 2% glucose) for 24 hr, diluted into YPA (YEP + 2% KAc) at OD_{600} = 0.2, and grown overnight. Cells were then resuspended in cold YEP (4°C) and elutriated as described in [42]. Cells were kept in cold media during the elutriation process and were then resuspended in SPO media to induce sporulation as described above.

Immunofluorescence Techniques

Indirect in situ immunofluorescence was carried out as described in [43]. Rat α-tubulin antibodies (Oxford Biotechnology) were used at a 1:100 dilution, and α-rat FITC or α-rat rhodamine antibodies (Jackson ImmunoResearch) were used at a 1:100 dilution. Mam1-9MYC was detected with a mouse α-MYC antibody (Babco) at a 1:250 dilution and an α-mouse FITC antibody (Jackson ImmunoResearch) at a 1:100 dilution. Metaphase I cells were defined as cells with one DNA mass spanned by a meiotic spindle measuring 2–3 μm in length. Anaphase I cells were defined as cells with spindles measuring at least 4 μm. Metaphase II and anaphase II cells were defined in a similar manner. Binucleated cells were defined as cells with two distinct but not necessarily separated DNA masses. Tetranucleated cells were defined in a similar manner.

Meiotic Spreads

Chromosomes were spread as described by [44]. Rec8-3HA was detected with a mouse α-HA antibody (Babco) at a 1:500 dilution and an α-mouse CY3 antibody (Jackson ImmunoResearch) at a 1:1000 dilution. Ndc10-13MYC was detected with a rabbit α-MYC antibody (Gramsch) at a 1:200 dilution and an α-rabbit FITC antibody (Jackson ImmunoResearch) at a 1:300 dilution. Mam1-9MYC was detected with a rabbit α-MYC antibody at a 1:50 and an α-rabbit FITC antibody at a 1:100 dilution. Ndc10-6HA was detected with a mouse α-HA antibody at a 1:200 and an α-mouse CY3 antibody at a 1:300 dilution. Lrs4-13MYC was detected with a rabbit α-MYC antibody at a 1:20 and an α-rabbit FITC antibody at a 1:100 dilution. Sgo1-9MYC was visualized with a rabbit α-MYC antibody at 1:150 and α-rabbit FITC antibody at a 1:300 dilution.

Quantification of Mam1, Lrs4, and Sgo1 Localization at Kinetochores

Mam1, Lrs4, and Sgo1 were scored as localized to kinetochores when more than 50% of the foci colocalized with Ndc10 foci on chromosome spreads.

GFP Chromosome Dots

Heterozygous CENV GFP dots were constructed from the integration of an array of bacterial TET operator sites 1.4 kb from the centromere of one homolog of chromosome V [19]. A TET repressor GFP fusion was also expressed in this strain so that the location of these TET operator sites would be marked with GFP. For visualization of these GFP-labeled chromosomes, cells were fixed in 3.7% formaldehyde for 10 min, then washed with 80% EtOH and resuspended in 1 mg/ml 4′,6-diamidino-2-phenylindole (DAPI) solution.

Western Blots

For preparation of extracts, cells were precipitated with 5% trichloroacetic acid (TCA) and lysed as described in [45]. Immunoblots were preformed as described in [46]. MYC tag proteins were detected with a mouse α-MYC antibody (Babco) at a 1:1000 dilution. Pgk1 was detected with a mouse α-PGK1 antibody (Molecular Probes) at 1:5000 dilution. The secondary antibody used was a goat α-mouse antibody conjugated to horseradish peroxidase (HRP; Jackson ImmunoResearch) at a 1:5000 dilution.

Chromatin Immunoprecipitation

Chromatin immunoprecipitations were performed as described in [47] with the following modifications. A monoclonal mouse α-HA antibody (12CA5; Roche) and a polyclonal rabbit α-Cep3 antibody (gift from Peter Sorger) were used (Cep3 is a component of the kinetochore). Cells (50 ml; OD_{600} = 1.9) were harvested 4 hr after transfer to sporulation medium and crosslinked with 1% formaldehyde for 40 min at room temperature. Centromeric primer sequences (L3, CEN3, R3) were described in [20]. Chromosome arm primers (approximately 80 kb from CENIII) are as follows: ARM-F, 5′-TCT TCTGGATTCCATGACAGA-3′; ARM-R, 5′-GGTTTTACAACAAAAGGT GGC-3′. PCR amplification was performed with a 30 cycle program (30 s at 94°C, 30 s at 52°C, and 60 s at 72°C) with input DNA diluted 1:500 with respect to immunoprecipitated samples. PCR products were resolved on 2% TBE-agarose gels and imaged with Alpha Imager software.

Supplemental Data

Supplemental figures are available with this article online at http://www.current-biology.com/cgi/content/full/14/24/2168/DC1/.

Acknowledgments

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